METHYLATED ALBUMIN CHROMATOGRAPHICAL PURIFICATION OF STREPTOLYSIN S SAMPLE*

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Since 1939, when the streptolysin S inducing effect of ribonucleic acid was found by H. Okamoto[1], many researchers [2)-7)] have devoted themselves to the study of isolation and purification of streptolysin S. And, it became now possible to obtain partially purified streptolysin S samples, the hemolytic potency of which may be considerably greater than any other hemolytic agent that we are aware.

However, no one has yet succeeded in isolating the lysin in the really pure form. The purified streptolysin S samples so far obtained are thought to be contaminated with ribonucleic acid originally added to the media, inactivated streptolysin S and a little amounts of other contaminants.[8)]

The problem of the nature of streptolysin S, therefore, awaits further study.

Recently, it was demonstrated in our laboratry that methylated albumin chromatography introduced by Mandel and Hershey[9)], and modified by Sueoka and Cheng[10)], could be very successfully utilized for further purification of the partially purified streptolysin S sample.

The present communication deals with the results obtained in such experiments.

Materials and Methods

Streptolysin S : Streptolysin S sample was prepared according to Okamoto et al.[2)] and Shoin[3)] from 30-hour culture of *Streptococcus hemolyticus*, strain "S", grown on meat-infusion broth containing 1% yeast ribonucleate. The so-called I-N-F streptolysin S fraction thus obtained was used throughout the experiments. The hemolytic activity of the sample was 100 hemolytic units per optical density at 260 mμ (or 1:5 millions, in a weight volume ratio).

Methylated albumin, buffered saline and methylated albumin coated kieselguhr

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(*MAK*) : These were prepared as described by Mandel and Hershey[9]

MAK column : The chromatographic column, which was comoposed of MAK, was prepared according to Sueoka and Cheng[10]. Usually 10 ml of MAK was packed under air pressure, the height of the packed material being 1.5 cm. The column was washed with 100 ml of 1.0 M buffered saline, then washed further with 100 ml of 0.05 M phosphate buffer pH 6.7, and equilibrated with enough volume of starting buffer or buffered saline. The column, after use, was regenerated according to this procedure and was safely used repeatedly.

Estimation of ribonucleotide and protein : The amount of ribonucleotide was estimated from the optical density at 260 mμ, and protein by the method of Lowry et al.[11]

Estimation of hemolytic activity : The streptolysin solution was diluted with physiological saline serially. To 1 ml of the diluted solution, 1 ml of 3 % rabbit erythrocytes suspension in physiological saline was added. After 30 minutes incubation at 37°C, 2 ml of physiological saline were promptly added and rapidly centrifuged. The resulting supernatant was measured for its optical density at 541 mμ with a spectrophotometer. The hemolytic unit (H. U.) was expressed as the multiple dilution value which produced 50 % hemolysis.

Experimental results

In order to obtain some information on the properties of hemolytic active substance, chromatographical behavior of the partially purified streptolysin S sample (I-N-F) was first investigated.

A partially purified sample (I-N-F) of streptolysin S was fractionated on 10 ml MAK-column (1.5 cm × 1.5 cm) employing the similar system as described by Sueoka and Cheng[10] : 1 mg of streptolysin S was dissolved in 50 ml of 0.05 M phosphate buffer pH 6.7 and charged on a column which had previously been equilibrated with the same buffer. Stepwise elution was carried out with increasing salt concentration of the buffered saline.

Five 5 ml fractions were collectd in each step at a flow rate of 5 ml per 3 minutes.

The elution pattern of this preliminary experiment was shown in Fig. 1. Attention should here be paid to following three points :

1) Of ultraviolet-absorbing material contained in the sample, 57 per cent passed through without being adsorbed on the column, and that this effluent was tested to be non-hemolytic.

2) Some ultraviolet-absorbing but not hemolytic material was also eluted from the column by 0.1~0.3 M NaCl.

3) Almost all the hemolytic active substance of the sample was found in the 0.4~0.6 M NaCl eluates.

On the basis of the above results, a chromatographical method consisted of two steps was developed for further purification of streptolysin S sample.

I. First chromatography

16 mg of streptolysin S sample was dissolved in 50 ml of 0.2 M buffered saline and applied in the same column used in the former experiment. The column, after washing with 20 ml of 0.2 M buffered saline, was eluted with 1.0 M buffered saline. All these operations were finished within 30 minutes at room temperature.

A sharp separation of non-hemolytic, ultraviolet-absorbing material from the streptolysin S sample could be expected, as may be seen from Fig. 2 and Table 1.

II. Second chromatography

The 1.0 M eluate obtained in the first chromatography, after diluted with 0.05 M phosphate buffer to the NaCl concentration of 0.05 M, was chromatographed on the same MAK-column with increasing salt concentration of the buffered saline in the same manner as described in the preliminary experiment. The result was presented in Fig. 3. The hemolytic unit per optical density at 260 mμ of the 0.5 M NaCl eluate, the highest potency one, was about 6×10^3. This value was, therefore, 60 times higher than that of the starting material, I-N-F streptolysin S fraction (cf. Table 1).

Fig. 4 shows the ultraviolet-absorption spectra of the 0.5 M NaCl eluate and that of the non-hemolytic effluent obtained in the first chromatography. The Folin-Lowry test has, however, indicated the presence of a small amount of protein in the 0.5 M NaCl eluate.

At any rate, it may be said that at least a 60-fold purification, based on optical density, of the partially purified streptolisin S sample could expected by the methylated albumin chromatography.

To be added here is the matter that quite similar chromatographical result was also obtained in the experiments performed with the partially purified streptolysin S sample[12] obtained from culture of hemolytic streptococci grown on 1 % RNase core*-broth.

Further study is now in progress.

Summary

Data was presented to show that methylated albumin column chromatography could be used for expecting a 60-fold purification of partially purified streptolysin S sample.

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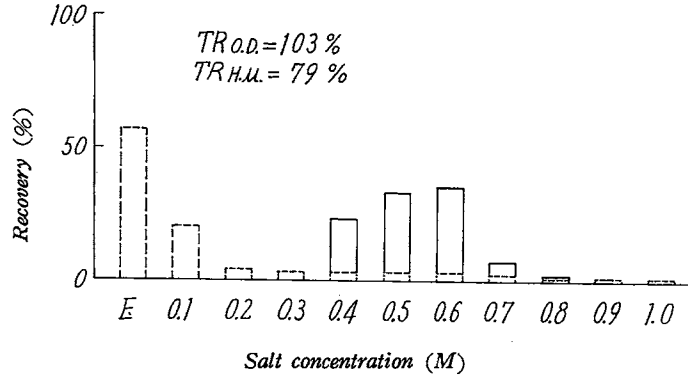
Fig. 1. Elution pattern of streptolysin S sample on MAK-column



Recovery of each salt concentration step was calculated from the sum of $OD_{260m\mu}$ and hemolytic unit in the five 5 ml fractions respectively. E: effluent, TR: total recovery

····· : $OD_{260m\mu}$; —— : H.U.

Fig. 2. First MAK-column chromatography of streptolysin S sample

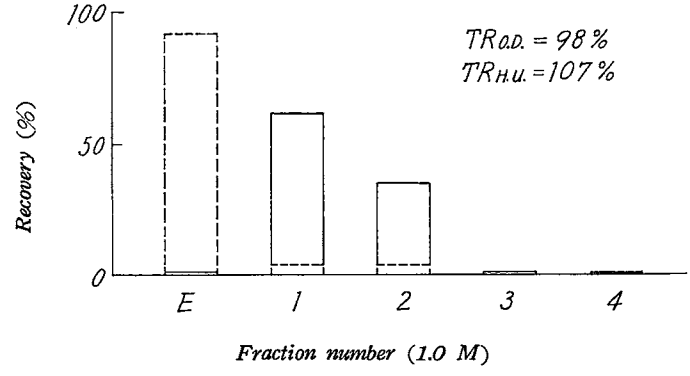
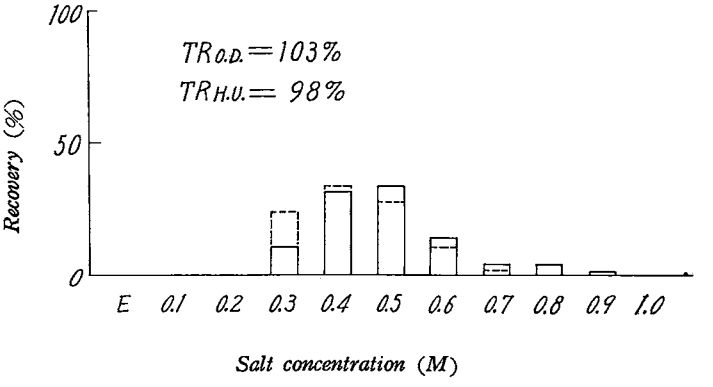


Table 1. First chromatography of streptolysin S sample on MAK-column

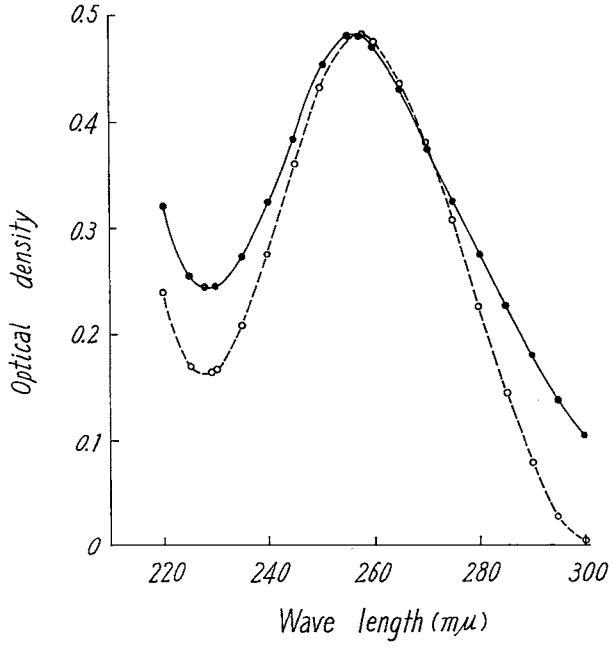
Fraction	Total OD260/fraction	Total H.U./fraction	H. U./OD260
Effluent	167.3	272	1.6
1.0 M-1	7.6	12368	1622.0
2	6.2	6944	1120.0
3	0	231	——
4	0	28	——
Original	185.3	18470	99.6

Fig. 3. Second chromatography on MAK-column of the 1.0 M NaCl eluate obtained in the first chromatography



7.3 OD_{260} units were charged.

Fig. 4. Ultraviolet absorption spectra



——: 0.5 M NaCl eluate obtained in the second chromatography

……: Effluent obtained in the first chromatography